The University of Chicago

ACTION OF SALTS UPON THE METABOLISM OF NERVES

A DISSERTATION
SUBMITTED TO THE FACULTY
OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY

BY
LLOYD KENDRICK RIGGS

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CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. XXXIX, No. 2
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Reprinted from The Journal of Biological Chemistry, Vol. XXXIX, No. 2, 1919

ACTION OF SALTS UPON THE METABOLISM OF NERVES.

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(Received for publication, July 7, 1919.)

INTRODUCTION.

The question of the relation of functional to metabolic activity in the nerve fiber has been made the subject of numerous investigations by Tashiro and his students. In the course of these investigations the following facts have been brought out.

1. Living nerve fibers give off carbon dioxide at rates which vary for various animals and for various nerves of the same individual (1).

2. The metabolic rate is not the same in all portions of a single nerve: Thus it has been shown that the rate of metabolism of a sensory nerve is highest in that portion of the nerve nearest to its peripheral ending and least near its central termination and that the reverse is true of a motor nerve (2, 3, 4).

3. Electrical stimulation of a nerve by means of a weak tetanizing current increases its rate of metabolism to about two and one-half times that of a resting nerve (1).

4. If a nerve is allowed to respire in an atmosphere of pure hydrogen the carbon dioxide production from it is found to be decidedly less than that of a similar nerve which has respired in an atmosphere containing oxygen. Further, electrical stimulation of a nerve in an atmosphere of hydrogen fails to bring about a marked increase of carbon dioxide production (5).

5. A 10° increase of temperature increases the rate of carbon dioxide production approximately 100 per cent (1, 6).

6. The treatment of a nerve with small concentrations of the narcotic drugs ethyl urethane and chloral hydrate brings about a state of increased irritability and corresponding with it a condition of increased rate of carbon dioxide production. A treatment with greater concentrations of these same drugs or with small concentrations for a longer period of time brings about a condition of depressed irritability or narcosis and with it a decreased rate of carbon dioxide production (7).

7. There is evidence for the general statement that nerves which conduct an impulse rapidly have a higher rate of metabolism than those which conduct at a lesser rate (3).

The interpretation which has been built up from these and similar facts is that the functional activity of the nerve is fundamentally a dynamic, metabolic process. If this interpretation is correct, we may then predict that any physical or chemical treatment which serves to throw a nerve into functional activity will be found also to increase its metabolic activity and that we may measure this, as was done in the experiments quoted above, by the rate of carbon dioxide production.

The action of inorganic salts upon physiological conditions or states constitutes one of the frequently studied subjects of physiology. Most of the experiments which have been devised in an effort to throw light upon the mechanism of salt action fall into one of the three following classes. First, experiments in which the living organisms or tissues are treated with solutions of various salts and observations are made upon the physiological effect of such treatment. Second, the effect of salts upon various processes and conditions such as speed of oxidation, surface tension, etc., has been studied *in vitro* and the results of such studies have been carried over to explain, by analogy, the physiological action of salts. The results of such studies are of a very considerable value, but there is always very grave danger that the conditions of the experiments *in vitro* are far from being represented in the living tissues. Third, a method of study has less frequently been undertaken in which the action of salts upon the functional condition of tissues has been studied simultaneously with some other process occurring within the tissues. This last is the type of study which has here been carried out and which is the basis of this report. The ideal material for such studies appeared to be the nerve on account of the fact that in such material the complicating factors of movement, cell division, secretion, etc., are absent, the only functional changes known to occur in the nerve being electrical and chemical changes.

The action of salts upon nerves has been carefully studied by Mathews (8) who has shown that when nerves are treated with salts a characteristic action (stimulation or depression) occurs, to a degree and within a period of time which is characteristic for each salt.

It was suggested to me by Professor Mathews that I undertake, under Professor Tashiro's direction, a study of the action of certain stimulating and depressing salts on the carbon dioxide production in nerve, in order to correlate their effect on metabolism and irritability.

I am much indebted to Professor Mathews for thus suggesting this problem.

EXPERIMENTAL.

Method.

The effect of salts upon the metabolism of nerves was determined by comparing the rate of carbon dioxide production of a nerve treated with a given salt solution, with that of a control nerve kept in Ringer's solution, such comparisons being made in Tashiro's biometer (9). Quantitative determinations were also made upon the carbon dioxide production of nerves under various conditions by means of his Apparatus III (10).

No doubt the ideal way of determining the effect of a salt upon the metabolism of a nerve would be a device for measuring the carbon dioxide produced during the time that the nerve is being bathed in a given salt solution, but on account of the fact that the presence of even a minute quantity of an aqueous solution in the chamber of the apparatus introduces a serious error into the determination of carbon dioxide, the following procedure was adopted.

A frog was killed by decapitation, a nerve muscle preparation quickly made, and hung in a moist chamber with the nerve dipping into the salt solution to be studied and the muscle attached to a lever writing on a slowly revolving drum. Stimulation of the nerve by the salt solution is then indicated by the twitching of the attached muscle. To study the carbon dioxide production of a nerve so treated it was cut free from the muscle and after the removal of all adherent solution by means of filter paper it was placed in the respiration chamber of the carbon dioxide apparatus.

Of course the physiological condition of a nerve at the time of its removal from the salt solution gives us very little evidence as to its physiological state during the time that it remains in the

respiration chamber, so that in order to determine this point controls were run in which the condition of the removal of the nerve from the salt solution to the respiratory chamber were duplicated as nearly as possible, except that the nerve was not cut free from the muscle. This was accomplished by lifting the nerve carefully from the salt solution, removing adherent solution by means of filter paper, and allowing it to remain in the moist chamber on a clean glass cover-slip. The results of these control experiments as well as the results of the determinations of carbon dioxide are given under the discussion of each salt.

Sodium Chloride.—When the sciatic nerve of a frog is treated in the manner described above with an $\frac{}{8}$ solution of sodium chloride, which is isotonic with frog blood, there is no obvious effect for a latent period of about 120 minutes. At about the end of this latent period a few twitchings indicate that the nerve is being stimulated by the salt solution. These twitchings are rhythmical and become gradually more vigorous and may persist for as long as several hours (7). If the nerve is removed to a glass plate within the moist chamber at any time during the latent period there is no indication of any stimulation of the nerve, but if it is removed from the solution after the stimulation has begun there is a period of about 2 or 3 minutes in which all twitchings stop, except those which are due to the handling of the nerve. Following this second short latent period there occurs a short period of vigorous stimulation which persists for from 1 to 4 minutes.

In the series of experiments which are summarized in Table I the carbon dioxide production from nerves kept in the sodium chloride solution for various periods of time was compared with that of control nerves kept in Ringer's solution for similar periods of time. In these experiments equal weights of the two sciatic nerves were placed in the sodium chloride and Ringer's solutions and at the end of the various periods of time indicated in the table their relative rates of carbon dioxide production were determined in the biometer. For the periods of time up to 135 minutes there was no detectable difference in the rates of carbon dioxide production from the two nerves, but for longer periods of time there was noted a slight but certain difference in favor of the nerve kept in the solution of sodium chloride. These ex-

TABLE I.

Comparison of CO_2 Production of Two Nerves, One Kept in $\frac{N}{8}$ NaCl, the Other in Ringer's Solution.

Nerve A. Time in $\frac{N}{8}$ NaCl.	Nerve B. Time in Ringer's.	Number of experiments.	Result.
min.	*min.*		
1	1	1	A = B
2	2	2	A = B
2	2	2	A > B
2	2	1	A < B
5	5	4	A = B
5	5	1	A < B
10	10	5	A = B
10	10	3	A > B
10	10	3	A < B
15	15	5	A = B
15	15	1	A > B
20	20	4	A = B
24	24	1	A > B
25	25	3	A = B
25	24	1	A = B
30	30	3	A = B
30	30	1	A > B
40	40	3	A = B
40	40	1	A > B
40	40	1	A < B
44	44	1	A = B
55	55	2	A = B
60	60	1	A = B
70	70	2	A = B
80	80	1	A = B
90	90	1	A = B
100	100	1	A = B
114	115	1	A = B
135	135	1	A = B
135	135	2	A > B
139	139	1	A = B
141	141	3	A > B
150	150	1	A > B
160	160	2	A > B
180	180	2	A > B

experiments indicate that there is an increased production of carbon dioxide from a nerve treated with sodium chloride, in isotonic solution, and that this period of increased metabolic activity comes on at a time which corresponds to the period of stimulation by the salt. In order to test further the actual constant occurrence of this difference as well as its relation to the period of stimulation, a second series of experiments was tried in which a nerve muscle preparation was suspended in a moist chamber and the nerve was allowed to dip into the solution of sodium chloride until the period of stimulation, as evidenced by the vigorous contractions of the muscle, was well developed. During this period of stimulation, the stimulated and the control nerve were re-

TABLE II.

*Comparison of CO_2 Production from a Nerve (B) Stimulated by an $\frac{N}{8}$ Solution of NaCl with That of a Control Nerve (A) in Ringer's Solution.**

Number of experiments.	Result.
6	A > B
2	A < B
1	A < B(?)
6	A = B

* Time not recorded.

moved from the solutions to the biometer and their rates of carbon dioxide production compared (Table II). In this series of experiments more time elapsed between the removal of the nerves from the solutions and the determination of their carbon dioxide production than in the first series, owing to the necessity of bringing the nerves to equal weight.

This series of experiments is rather less convincing than the first, for in none of them was there more than a slight difference in the rate of carbon dioxide production noted and out of fifteen experiments only six confirmed the conclusion suggested by the first series. However, in view of the short duration of the period of stimulation following the removal of the nerve from the salt solution, it appears that the reason for this discrepancy in results might be that a greater length of time is required in the second series for the transfer of the nerves from the solution to the

biometer. In order to test this possibility a third series of experiments was carried out in which a nerve was kept in a solution of sodium chloride until it was being vigorously stimulated by the salt solution. It was then removed from the solution to the biometer, a control nerve kept in Ringer's solution being placed in the other. The nerves were weighed after the experiment was complete and the results recorded only for those experiments in which there was a difference of but a few mg. in the weight of

TABLE III.

*CO_2 Production of a Nerve Stimulated by $\frac{N}{8}$ NaCl Compared with That of a Control Nerve.**

Nerve A, being stimulated by $\frac{N}{8}$ NaCl; Nerve B, kept in Ringer's solution.

Weight of Nerve A.	Weight of Nerve B.	Number of experiments.	Result.
mg.	*mg.*		
24	26	1	A > B
26	25	1	A > B
23	28	1	A = B
17	16	1	A > B
20	21	1	A > B
22	21	1	A > B
30	29	1	A > B
25	25	1	A > B
24	22	1	A = B
19	18	1	A = B
(?)†	(?)†	4	A > B

* The nerves were weighed after the CO_2 production had been compared.

† The nerves in these experiments were of the same weight but the weight was not recorded.

the nerves (Table III). In this series of fourteen experiments eleven show a greater production of carbon dioxide by the nerve stimulated by means of the sodium chloride solution. This series of experiments taken together with those of the first series (Table I) indicate clearly that a period of increased carbon dioxide production occurs in a nerve coincident with a period of stimulation by an isotonic solution of sodium chloride.

No quantitative determinations were made on the carbon dioxide production by nerves treated with sodium chloride

solution, on account of the fact that the period of stimulation following the removal of the nerve from the salt solution persists for so short a time, certainly less than that required for respiration in the chamber of the quantitative apparatus.

Although quantitative determinations were not made, there can be no doubt there is an increase in the rate of carbon dioxide production from a nerve treated with an isotonic solution of sodium chloride for periods of about 130 or 150 minutes. This period of increased metabolic rate corresponds to a short period of increased functional activity of the nerve fiber which follows the removal of the nerve from the salt solution.

Sodium Sulfate.—While the experiments with sodium chloride indicate that the metabolic activity of a nerve is increased during salt stimulation, we are given no clue as to the extent of such increase. It is of interest to know if this increase is of the same order as that due to electrical stimulation. It therefore appeared to be of interest to select a salt which would not only stimulate a nerve, but one in which the period of stimulation would continue for a sufficiently great length of time after the removal of the nerve from the solution to enable quantitative determinations to be made during this period of stimulation. After the trial of several salts sodium sulfate was found to satisfy these conditions.

When a nerve muscle preparation is hung in a moist chamber with the nerve dipping into an isotonic ($\frac{N}{8}$) solution of sodium sulfate, a series of events occurs which is very similar to that occurring in the case of sodium chloride, except that the latent period is only 20 minutes in length and the contractions which follow the latent period are stronger and so rapid as to blend into a long low tetanus. When a nerve is removed from the solution and placed on a glass plate within the moist chamber, at any time during the latent period, no contractions occur (except in a few cases which will be noted later). If, however, the nerve is similarly removed from the solution during the period of salt stimulation, there follows after a second latent period of 2 or 3 minutes, a high tetanus which persists for about 35 or 45 minutes (Fig. 1).

A series of experiments was carried out in which the two sciatic nerves of a frog were removed as quickly as possible and placed, the one in an $\frac{N}{8}$ solution of sodium chloride and the other in an $\frac{N}{8}$ solution of sodium sulfate. At the end of various periods

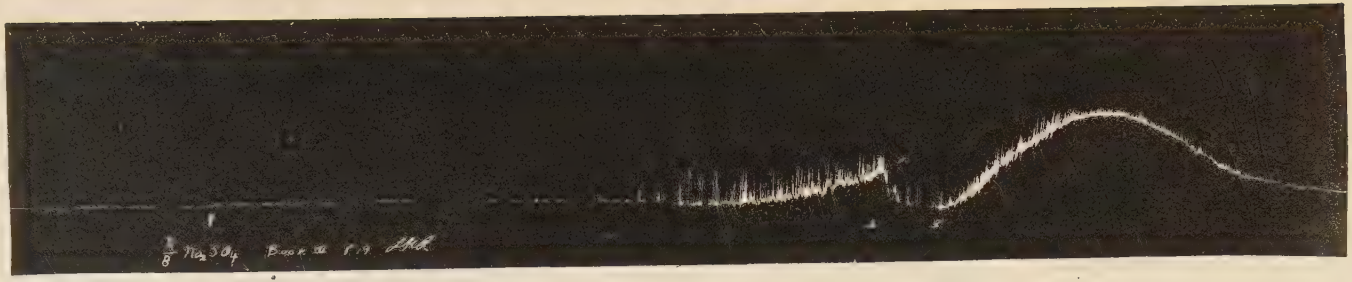

FIG. 1. 1. Latent period. Nerve bathed by $\frac{N}{8}$ Na_2SO_4.
2. Nerve removed from stimulating solution.
2 to 3. Latent period.
3. Beginning of second stimulation; *i.e.*, stimulation after removal from the $\frac{N}{8}$ Na_2SO_4.

TABLE IV.

Comparison of CO_2 Production of Two Nerves, One Kept in $\frac{N}{8}$ NaCl, the Other in $\frac{N}{8}$ Na_2SO_4.

Nerve A. Time in $\frac{N}{8}$ NaCl.	Nerve B. Time in $\frac{N}{8}$ Na_2SO_4.	Number of experiments.	Results.
min.	*min.*		
1	1	2	A = B
1	1	1	A < B
2	2.5	1	A = B
5	5	2	A = B
4	4	1	A = B
8	8	1	A = B
10	10	4	A = B
15	15	2	A = B
20	20	3	A = B
20	20	1	A < B
25	25	1	A = B
25	25	3	A < B
30	30	1	A < B
30	30	1	A = B
40	40	2	A < B
45	45	1	A = B
55	55	1	A = B
55	55	2	A < B
60	60	2	A = B
60	60	1	A < B
70	70	1	A > B
70	70	1	A < B
80	80	2	A = B
95	95	1	A = B
95	95	1	A < B
100	100	2	A = B
110	110	1	A > B
114	114	1	A = B
120	120	1	A = B
120	120	1	A > B
140	140	2	A > B
180	180	1	A > B
240	240	1	A = B
300	300	1	A = B

of time these nerves were removed from the solutions and their carbon dioxide production was compared in the biometer (Table IV).

For periods of time up to about 25 minutes there is no constant difference in the rate of carbon dioxide production from the two nerves, but from this time up to about 70 minutes there is a greater production of carbon dioxide from the nerve kept in the solution of sodium sulfate. There is again no marked difference noted until the periods from 120 to 180 minutes, when there is a greater production of carbon dioxide from the nerve kept in the solution

TABLE V.

CO_2 Production of a Nerve Stimulated by $\frac{N}{8}$ Na_2SO_4 Compared with That of a Control Nerve in $\frac{N}{8}$ NaCl or in Ringer's Solution.

Number of experiments.		Result.
Nerve A kept in $\frac{N}{8}$ NaCl.	Nerve B stimulated by $\frac{N}{8}$ Na_2SO_4.	
8	8	A < B
1	1	A = B
1	1	A = B(?)

Weight of Nerve A kept in Ringer's.	Weight of Nerve B stimulated by $\frac{N}{8}$ Na_2SO_4.	Number of experiments.	Result.
mg.	*mg.*		
30	30	1	A < B
25	23	1	A < B
24	26	1	A = B
24	25	1	A < B
31	29	1	A < B

of sodium chloride. These periods of increased metabolism have a general correspondence to the periods of stimulation by the salts used in these experiments.

In order to study further the relation between the period of increased metabolic activity and the period of stimulation by sodium sulfate, a second series of experiments was carried out in which a nerve muscle preparation was set up in the moist chamber with the nerve dipping into $\frac{N}{8}$ sodium sulfate. The nerve was removed and its carbon dioxide production compared with that

of a control nerve kept in Ringer's solution, after the period of stimulation was well developed (Table V).

In twelve out of fifteen of these experiments the carbon dioxide was found to be greater from the nerve which was being stimulated by the sodium sulfate than from the control nerve which had been kept in $\frac{N}{8}$ sodium chloride solution or in Ringer's solution. The results are the same in these experiments when the nerves are weighed before the experiment as when they are weighed after the experiment is completed. These experiments show that the period of increased metabolic activity is also the period of stimulation by the salt.

The fact that the period of stimulation after the removal of the nerve from the salt solution persists for so long a time (35 to 45 minutes) makes it possible to make quantitative determinations of the production of carbon dioxide during the time that this period of stimulation still exists in the nerve. In order to determine the degree to which the production of carbon dioxide is increased by stimulation with sodium sulfate, a nerve muscle preparation was set up in the moist chamber and when the contractions of the muscle indicated that the nerve was being stimulated, it was removed to the respiration chamber of the quantitative apparatus.[1] The results of these determinations are given in Nos. 7, 8, 9, and 10 of Table VI.

These experiments show that the carbon dioxide production of a nerve stimulated by sodium sulfate is increased from 2.5×10^{-7} to 5.5×10^{-7} gm. of carbon dioxide per centigram of tissue for each 10 minutes of respiration; *i.e.*, the rate of metabolism of the nerve is increased 2.2 times by the salt stimulation. This increase, while it is less than that noted when a nerve is stimulated electrically (found by Tashiro (1) to increase the metabolism of a nerve 2.4 times) is of the same order of increase. That sodium sulfate stimulation is less intense than electrical is indicated by the fact that if an electrical stimulus just strong enough to be felt on the tongue is sent into the nerve at the height of the

[1] The respiration period used in these experiments was 30 minutes and as the nerves were kept in the solution from 20 to 30 minutes, the nerve had been out of the body of the animal for about 1 hour before the experiment was complete. This fact should be kept in mind when comparing these results with the quantitative results of Tashiro.

contractions due to the salt stimulation, the muscle responds with still higher contractions. In other words sodium sulfate stimulation is not maximal. This lesser grade of stimulation may be able to account for the fact that the metabolism of a nerve is not increased so much by sodium sulfate as by electrical stimulation.

The question as to the metabolic activity of a nerve during the latent period of sodium sulfate action appeared to be of special interest on account of the fact that it was frequently noted that

TABLE VI.

*Effect of $\frac{N}{8}$ Na_2SO_4 on the Sciatic Nerve of the Frog.**

No.	Solution.	Time in solution.	Condition of nerve.	CO_2 produced by 10 mg. of nerve in 10 min.	Range of temperature.
		min.		*gm.*	*°C.*
1	$\frac{N}{8}$ Na_2SO_4	5	Irritable.	4.1×10^{-7}	18–21
2	Ringer's.	5	"	4.3×10^{-7}	18–22
3	$\frac{N}{8}$ Na_2SO_4	10	"	4.1×10^{-7}	17–20
4	Ringer's.	10	"	3.8×10^{-7}	18–21
5	$\frac{N}{8}$ Na_2SO_4	15	"	3.0×10^{-7}	17.5–20
6	Ringer's.	15	"	2.8×10^{-7}	17.5–22
7	$\frac{N}{8}$ Na_2SO_4	20	Stimulated.	5.5×10^{-7}	17–20.5
8	Ringer's.	20	Irritable.	2.5×10^{-7}	19–21
9	$\frac{N}{8}$ Na_2SO_4	30	Stimulated.	5.4×10^{-7}	18–21
10	Ringer's.	30	Irritable.	2.4×10^{-7}	18–22

* Each entry in this table is the average of several determinations. All determinations were made at temperatures including the extremes given in Column 6 of this table.

a nerve was extremely irritable during and especially near the end of the latent period. This heightened irritability was at times so great that the slamming of a door would cause a contraction of the muscle. In an effort to throw light upon this question, the carbon dioxide production of nerves during various portions of the latent period was studied quantitatively and the results of these determinations compared with the results of studies similarly made upon control nerves kept in Ringer's solution. Several complicating factors have, however, rendered these results unsatisfactory. In the first place nerves do not always show this increased irritability in the latent period, secondly it

does not appear that this state of heightened irritability always remains after the nerve has been removed from the salt solution, and finally some exceptions were noted to the general rule that a nerve removed from the solution during the latent period does not show signs of stimulation. The results of these experiments are given in Table VI. It has been as yet impossible to control the above complicating factors sufficiently well to warrant any conclusion other than, that for periods of time up to 15 min-

TABLE VII.

Comparison of CO_2 Production of Two Nerves, One Kept in $\frac{N}{8}$ KCl, the Other in $\frac{N}{8}$ NaCl or in Ringer's Solution.

Time of Nerve A in $\frac{N}{8}$ NaCl.	Time of Nerve B in $\frac{N}{8}$ KCl.	Number of experiments.	Result.
min.	*min.*		
5	5	2	A > B
10	10	1	A > B
15	15	1	A > B
22	22	1	A > B
30	30	1	A > B
40	40	2	A > B
In Ringer's.			
10	10	2	A > B
15	15	2	A > B
20	20	1	A > B
30	30	1	A > B
40	40	1	A > B
	Stimulated electrically.		
15	15	3	A > B
20	20	1	A > B
30	30	3	A > B

utes, a nerve in an isotonic solution of sodium sulfate has a rate of respiration not markedly different than that of a control nerve kept in Ringer's solution.

Potassium Chloride.—When a nerve is treated with an isotonic solution ($\frac{N}{8}$) of potassium chloride its irritability is speedily abolished so that it does not even respond to the strong stimulus of cutting. This non-irritable or narcotized state persists after the nerve has been removed from the solution but its normal condi-

tion returns when it is placed in sodium chloride or in Ringer's solution. That this depression of irritability is accompanied by a decrease of metabolic activity is shown by a series of experiments in which the carbon dioxide produced by a nerve so treated is compared with that of a nerve of the same animal kept for the same length of time in an $\frac{N}{8}$ solution of sodium chloride or in Ringer's solution. Electrical stimulation of a nerve which has been rendered non-irritable by means of potassium chloride fails to increase markedly its rate of carbon dioxide production (Table VII).

There is no primary increase in the rate of carbon dioxide production, from a nerve treated with a solution of potassium chloride, such as was observed by Tashiro and Adams (7) in the

TABLE VIII.

*Effect of $\frac{N}{8}$ KCl on the Sciatic Nerve of the Frog.**

No.	Solution.	Time in solution.	Condition of nerve.	CO_2 produced by 10 mg. of nerve in 10 min.	Range of temperature.
		min.		*gm.*	*°C.*
1	$\frac{N}{8}$ KCl	5	Non-irritable.	1.9×10^{-7}	20–26
2	Ringer's.	5	Irritable.	4.3×10^{-7}	18–22
3	$\frac{N}{8}$ KCl	30	Non-irritable.	1.59×10^{-7}	20–24
4	Ringer's.	30	Irritable.	2.4×10^{-7}	18–22

* Each entry in this table is the average of several determinations. All determinations were made at temperatures including the extremes given in Column 6 of this table.

case of narcosis by drugs. This fact is interesting in view of the fact that Mathews failed to discover any primary stimulation of a nerve by an isotonic solution of potassium chloride.

The degree to which the metabolism of a nerve is depressed by an $\frac{N}{8}$ solution of potassium chloride has been studied quantitatively and the results are given in Table VIII.

These experiments show that a nerve treated with an $\frac{N}{8}$ solution of potassium chloride has its rate of carbon dioxide production decreased from 4.3×10^{-7} to 1.9×10^{-7} gm. of carbon dioxide per centigram of tissue for each 10 minutes respiration and that a treatment for 30 minutes decreases its rate of carbon

dioxide production from 2.4×10^{-7} to 1.5×10^{-7}. In other words a nerve kept in a solution of potassium chloride for 30 minutes has a rate of metabolism only 0.66 that of a normal nerve kept for the same length of time in Ringer's solution, and the carbon dioxide production of a nerve kept in the potassium chloride solution for 5 minutes is 0.44 that of a nerve kept in Ringer's solution for the same length of time. These results are of the same order as the results of Tashiro and Adams (7) who found that the carbon dioxide production from the claw nerve of the crab, *Labinia canaliculata*, which they rendered non-irritable by means of drugs, was reduced to from 0.53 to 0.35 that of the normal nerve.

SUMMARY.

The facts brought out in the preceding quantitative studies may be easily summarized in the curves of Fig. 2 in which the

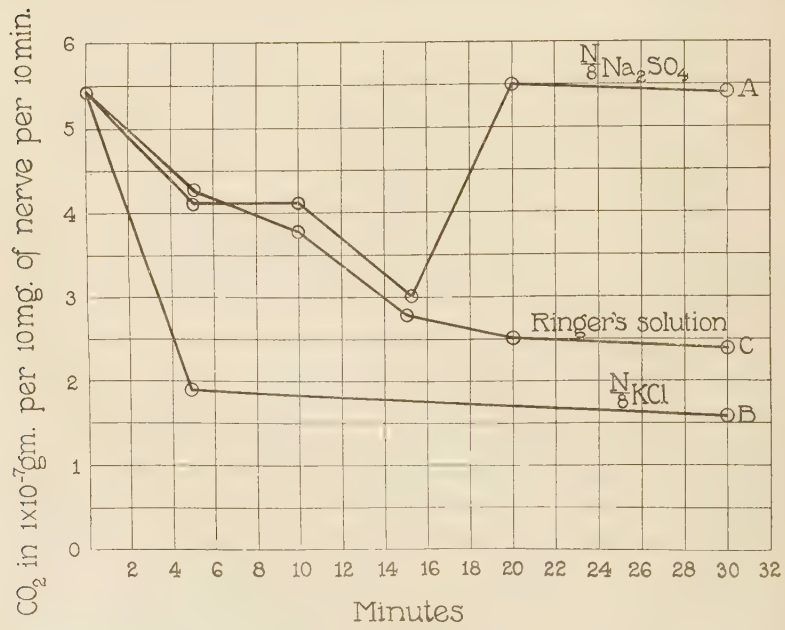

FIG. 2. Quantitative comparisons of CO_2 productions from the nerves treated by (*A*) $\frac{N}{8}$ Na_2 SO_4, (*B*) $\frac{N}{8}$ KCl, and (*C*) Ringer's solution.

carbon dioxide produced, expressed in gm. of carbon dioxide per cc. of tissue for each 10 minutes respiration, is represented by the ordinates and the time that the nerves are allowed to remain in the solution by the abscissæ.

The carbon dioxide production of a normal nerve suffers a gradual decrease in rate after the nerve is removed from the body and allowed to remain in Ringer's solution while that of a nerve kept in a solution of sodium sulfate isotonic with frog's blood suffers a similar decrease up to the time that it is stimulated by the salt solution, at which time a sudden increase in the rate of carbon dioxide production occurs. This increase in the rate of metabolism is to the extent of 2.2 times that of the resting nerve.

The increase in the metabolic rate of a nerve by sodium sulfate stimulation is of the order of that of electrical stimulation and indicates that the mechanism involved is the same in both cases and is an additional indication that the relation between stimulation and increased rate of metabolic activity is a causal one or at least that an increased rate of carbon dioxide production is an invariable accompaniment of increased functional activity.

Additional evidence is found in these results against the view of Bayliss (11), who holds that the increased production of carbon dioxide observed when a nerve is stimulated by means of an electrical current may be due not to increased metabolic activity of the nerve fiber but rather to the heating of the nerve by the electrical current. In these experiments there is no obvious means of heating the tissue, or at least there appears to be no greater possibility of heating a nerve by means of an isotonic solution of sodium sulfate than by a similar solution of potassium chloride or by Ringer's solution.

The action of potassium chloride in abolishing the irritability of a nerve and at the same time reducing its metabolic rate is in accord with the broad general thesis of the relations between functional and metabolic activities. The effect of potassium chloride upon the metabolism of nervous tissue rendered non-irritable by its action is similar to that of the drugs studied by Tashiro and Adams and lends further support to the view developed chiefly from the findings of Mathews, Loevenhart, and Verworn that the primary effect of the narcotic agents, drugs, or

salts is upon the chemical, metabolic activity of the living substance.

The suggestion that biological "salt action" in general is upon the chemical activity of the living substrate is supported by this paper.

To Dr. Shiro Tashiro, under whose direction these experiments have been carried out, I wish to express my sincere gratitude.

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